MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2021

January–March 2021—Volume 136, pp. 215–218
https://doi.org/10.5248/136.215

Dichotomophthora portulacae, a new record from Iraq

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ABSTRACT—*Dichotomophthora portulacae* isolated from *Portulaca oleracea* is newly recorded from Iraq. The ITS sequence showed congruence with *D. portulacae* sequences deposited in GenBank. A phylogram, description, and illustrations are provided.

KEY WORDS—conidial fungi, taxonomy, plant pathogenic fungi

Introduction

Dichotomophthora was described by Mehrlich & Fitzpatrick with the single species *D. portulacae* isolated from *Portulaca oleracea* in Hawaii. *Dichotomophthora portulacae* causes diseases on the succulent weed purslane, *Portulaca oleracea* (Mehrlich & Fitzpatrick 1935, Ellis 1971). The fungus produces a necrotic leaf spot and dark lesions on stems (Klisiewicz 1985, Klisiewicz & al. 1983).

Materials & methods

Fungal isolation, culture, and observation

Samples were collected from *Portulaca oleracea* in Basra Governorate, Iraq. Pieces of stems and leaves with disease symptoms were surface disinfected in 3% sodium hypochlorite for 5 min, rinsed in sterile distilled water, and plated on water agar. Hyphae originating from these tissue pieces were transferred to potato dextrose agar (PDA). Morphological characteristics such as conidiophore length, conidial dimension, and sclerotial dimension were measured at 40× magnification. Cultural characteristics such as colony appearance, conidial shape, colour and shape of sclerotia over the plate were also examined. The living culture, slant tube, and dried agar plate are conserved in the author's private herbarium "herb. Z.K. Abdulla."

DNA extraction, amplification, and sequence analysis

A monoconidial culture was grown on PDA for 10 days at 27 °C and mycelium was harvested by scraping the culture using a sterile scalpel. Genomic DNA was extracted from mycelium by using Ez-10 Spin Column Fungal Genomic DNA Mini-Preps Kit. PCR amplification was performed in a 50 µl total volume containing 10 µl DNA template, 25 µl master mix, 11 µl nuclease-free water, and 2 µl of ITS 1 and ITS4 primers (White & al. 1990). PCR was performed in an Eppendorf Thermal Cycler following the protocol: 1 cycle of 3 min denaturation at 94 °C, then 35 cycles of 40 sec at 94 °C, 60 sec at 55 °C, 60 sec at 72 °C, and ending with one 10 min final extension at 72 °C. The amplified product was checked by electrophoresis on 2% agarose gel in 1 × TBE (trisBorate) buffer stained with ethidium bromide (1mg/L) and visualized under UV light.

For sequencing, the PCR product was purified with Gen All in One Combo Kit according to the manufacturer's instructions and sequenced by Macrogen using with ABI prism 3700 sequencers. The sequences were analysed using DNA Dragon (http://www.sequentix.de/dnadragon.) and GenBank's BLAST program (http:/blast.ncbi.nlm.nih.gov).

Taxonomy

Dichotomophthora portulacae Mehrl. & Fitzp. ex M.B. Ellis,
Demat. Hyphomyc.: 388 (1971) FIG. 1

COLONIES on PDA were effuse, greyish brown, hairy, or velvety, producing red pigment into the media. Mycelium was superficial and sometimes immersed, composed of smooth, branched, brown hyphae. SCLEROTIA spherical to globose or irregular, dark brown or black non-solid 180–280 × 180–220 µm. CONIDIOPHORES macronematous, mononematous, erect, cylindrical below, dichotomously or trichotomously branched, brown or hyaline, branches ≤400 µm long, 6–10 µm thick. CONIDIOGENOUS CELLS polytretic, lobed, discrete, determinate. CONIDIA solitary, cylindrical clavate, straight, round at the ends, smooth or verruculose, 17.5–55 × 10–15 µm, 1–4-septate, hyaline or pale brown, with a dark scar at the conidiogenous locus.

SPECIMEN EXAMINED: **IRAQ, BASRAH PROVINCE, Al Jazeera:** on stems and leaves of *Portulaca oleracea* L. (*Portulacaceae*), 15 Mar 2014, coll. Z.K. Abdulla (herb. Z.K. Abdulla ZKA1; strain ZAI1; GenBank MN508373).

Discussion

The Iraqi *Dichotomophthora portulacae* isolate resembled that described by Ellis (1971) except that the Iraqi conidia were smaller with 1–4 transverse

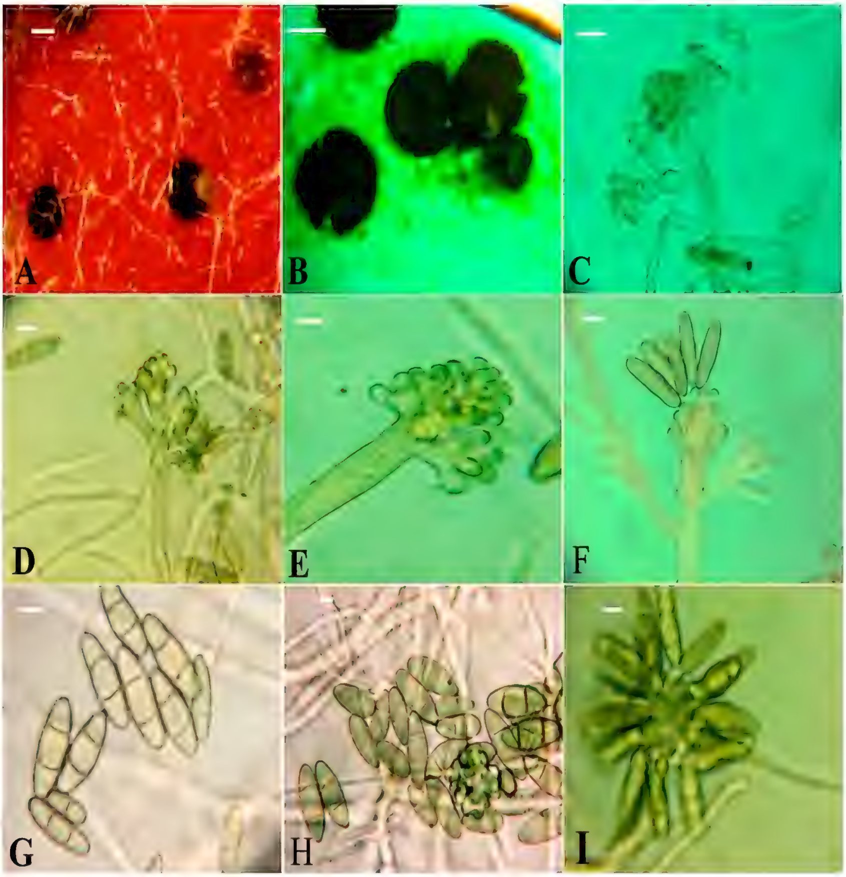

FIG. 1. *Dichotomophthora portulacae* (herb. Abdulla ZKA1). A, B. Sclerotia; C, D. Branched conidiophores; E. Lobed conidiophore; F–I. Conidia. Scale bars: A, B = 100 µm; C–I = 10 µm

septa and the conidiophores were usually dichotomously or trichotomously branched. The cultures produced pigment that converted the PDA to scarlet red after a few days. The morphological characters otherwise matched those of *D. portulacae,* and the culture's ITS sequence confirmed this identification. *Dichotomophthora portulacae* was isolated by Eken (2003) from *Portulaca oleracea* in Turkey and by Jing & al. (2008) from *Basella rubra* L. in China. The *Dichotomophthora* sp. isolated by Soares & Nechet (2017) from *Anredera cordifolia* (Ten.) Steenis in Brazil was identified based on morphology as

D. lutea; analysis of its ITS sequence showed high homology with a GenBank sequence labelled as "*Bipolaris* sp."

Acknowledgments

The author is truly grateful to Dr. Rafael F. Castaneda-Ruiz and Dr. Eric McKenzie for reviewing the paper and I give them all the respect and thanks for their helpful suggestions; also I thank Dr. Lorelei L. Norvell, Editor-in-Chief, and Dr. Shaun Pennycook, Nomenclature Editor, Mycotaxon for their efforts.

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